CELLULAR MECHANISMS OF HIGH-FREQUENCY ALTERNATING CURRENT BLOCK IN PERIPHERAL NERVES

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CELLULAR MECHANISMS OF HIGH-FREQUENCY ALTERNATING CURRENT BLOCK IN PERIPHERAL NERVES

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LIST OF SYMBOLS AND ABBREVIATIONS

HFAC	High-frequency alternating current
TTX	Tetrodotoxin
PDMS	Polydimethylsiloxane
CAP	Compound action potential
PDMS	Polydimethylsiloxane
Hz	Hertz
kHz	Kilohertz
mA	Milliampere
V	Volt
mV	Millivolt
µV	Microvolt
s	Second
ms	Millisecond

ABSTRACT

High-frequency alternating currents (HFAC) can be applied to nerves to reversibly stop the conduction of signals in peripheral nerves. This can be useful in treating conditions such as chronic pain, inflammation, and neuromuscular pathologies where there is excessive neuronal activity which can cause decreased motor control or painful sensations. However, the cellular mechanisms underlying HFAC block is not well understood. In this study, tetrodotoxin (TTX), a sodium channel blocker whose cellular mechanism is known is utilized to examine the cellular mechanisms of HFAC. We expect low dosages of TTX to alter the threshold of the HFAC required for complete block. Understanding how HFAC induces block can affect how we treat neuropathologies by this technique.

CHAPTER 1

INTRODUCTION

The ability to block activity of peripheral nerves has many useful implications in eliminating symptoms of chronic pain, spasticity, dystonia, and inflammation. In these pathological conditions, an excess of neuronal activity occurs creating uncomfortable/painful sensations as well limited motor control in the case of neuromuscular pathologies. Therefore, it is helpful to halt these signals. Many methods of block have been utilized including pharmacological, thermal, and surgical techniques to employ a stop to signal propagation. However these are not viable options to be used chronically in patients due to the fact they cause irreversible blockage, are slow acting, cause non-localized effects, or causes nerve damage [1,2,3,4,5.]

One method that eliminates much of these problems associated with blocking nerve signals is the application of high-frequency alternating current (HFAC) waveforms. It has been shown that HFAC waveforms in the range of 1-50 kHz reversibly and quickly blocks nerve propagation completely [6,7,8,9.] The amount of block produced can be examined via motor response of muscle force in the animal or compound action potential (CAP) recordings produced by the nerve upon stimulation [10,11,12.] It has also been shown that myelinated and unmyelinated fibers within the nerve bundle respond differently with increasing frequencies of HFAC and have different thresholds. While unmyelinated and myelinated fibers have many similar characteristics, myelinated fibers require a decreasing amplitude for block to occur with frequencies greater than 12 kHz [13,14.] This nonmonotonic behavior of induced block in myelinated fibers illustrates the potential to specifically block fibers based on myelination.

Knowing HFAC block's potential to be used clinically is vital. However, an understanding of how HFAC works in terms of biophysical mechanisms is needed. It is

known that nerves signals are propagated through action potentials involving voltage-gated calcium, sodium, and potassium channels. These channels open and close in response to polarization changes. It is predicted that because current changes the voltage of the cells, these ion channels play a role in preventing the nerve signal from propagating. Computational studies as well as modeling studies have offered various possible mechanisms of how block is induced. Some modeling experiment results suggest that voltage gated potassium channels are constantly activated in HFAC induced block, whereas some other modeling studies suggest the deactivation of sodium channels involved in the depolarization phase of an action potential is responsible for the block [15,16,17,18.] Pharmacological studies have shown particular chemicals to block certain ion channels. Tetrodotoxin (TTX) is a chemical toxin derived from pufferfish that blocks sodium channels [19,20,21.] Therefore, in this study of elucidating how nerve signals are blocked via HFAC stimulation, TTX is utilized. By applying different concentrations of TTX to the nerve, we can see whether this modulates the minimum amplitude of HFAC required to block a nerve. This will demonstrate the role of sodium channels in the blocking mechanism of HFAC.

CHAPTER 2

MATERIALS AND METHODS

Set-up of nerve

Seven rat sciatic nerves with lengths ranging from 25mm-34mm were extracted for experimentation. Nerves was used immediately upon extraction to maintain viability and was placed in a Petri dish over a layer of PDMS. Sciatic nerves were bathed in Ringer's solution (7.2pH) and pinned down on each side to secure in place. Four suction electrodes with an inner diameter of 0.84mm were suctioned onto the nerve at equidistant distances from each other (Figure 1). Grounding electrodes for each suction electrode was submerged in the Ringers solution surrounding the nerve. Additionally, the Petri dish was grounded to the cage and the cage was grounded to the ground of the building.

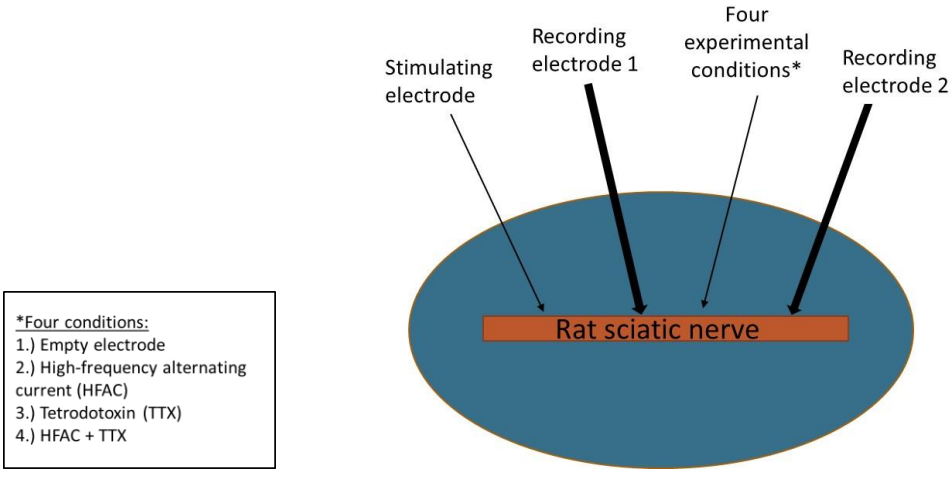


Figure 1: Experimental set-up of nerve and electrodes

Set-up of Equipment

A computer connected to a data acquisition (DAQ) board sent information to an electrode to stimulate the nerve and to another electrode to apply HFAC via two stimulus

isolators and a function generator. The DAQ board also received input from two recording electrodes on the nerve that record compound action potentials (CAP.) A differential amplifier was utilized to amplify signals by 10 or 100 fold as well as to filter certain frequencies.

Application of HFAC

High-frequency alternating current was applied to nerves to block nerve conduction. In each trial, a nerve was applied with a stimulation pulse of 5-10Vpp. The CAP elicited by the stimulation was recorded by the first recording electrode. A second recording electrode recorded the CAP in a location downstream to the application of HFAC (figure 1.) An HFAC waveform (5-50kHz, 0.1-10mA) was produced by a function generator and applied to the nerve via suction electrode. The frequency and amplitude of the waveform was adjusted depending on each nerve to achieve block. CAP recordings were taken every one minute.

Application of TTX

Tetrodotoxin was applied to nerves to block nerve conduction. Each trial consisted of a similar set-up to that of when HFAC was applied including an electrode stimulating the nerve (5-10Vpp) and two recording electrodes. In place of the electrode applying HFAC, a needle with 10µl (5nM) TTX was injected intrafascicularly into the nerve. TTX was allowed to incubate in the nerve for 10 minutes prior to beginning recording CAP's every one minute. After 10 recordings, another 10µl (5nM) TTX was injected to increase the concentration of TTX in the nerve. This was repeated approximately four times for one nerve.

CHAPTER 3

PRELIMINARY RESULTS

Approximately 500 recordings were taken from seven rat sciatic nerves to observe changes in action potential conduction. Recordings were made for the application of stimulus, TTX, and HFAC.

Stimulus artifact and nerve response

Application of a stimulation pulse of 5vPP to the rat sciatic nerve is seen in the stimulus artifact (Figure 2). In all trials, three runs were recorded one after the other with the average of all three plotted (Figure 2b, c). In some trials, the stimulation also elicited a nerve response as shown in the CAP recording from the recording electrode (Figure 3).

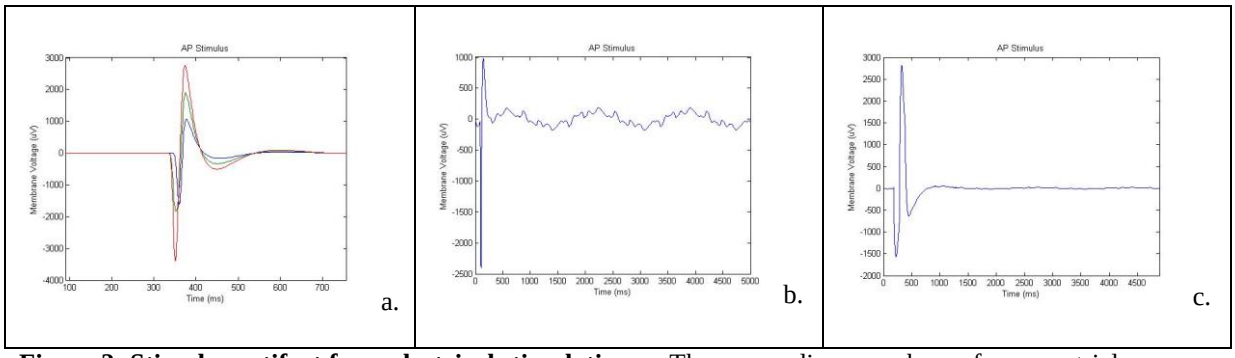


Figure 2: Stimulus artifact from electrical stimulation. a. Three recordings are shown from one trial, bandwidth: 300Hz/500Hz, sampling rate: 10µs, gain: 100. b. Average recording of 3 runs with noise present, bandwidth: 300Hz/1kHz, sampling rate: 10µs, gain: 1000. c. Average recording of 3 runs with reduced noise, bandwidth: 300Hz/1kHz, sampling rate: 10µs, gain: 1000.

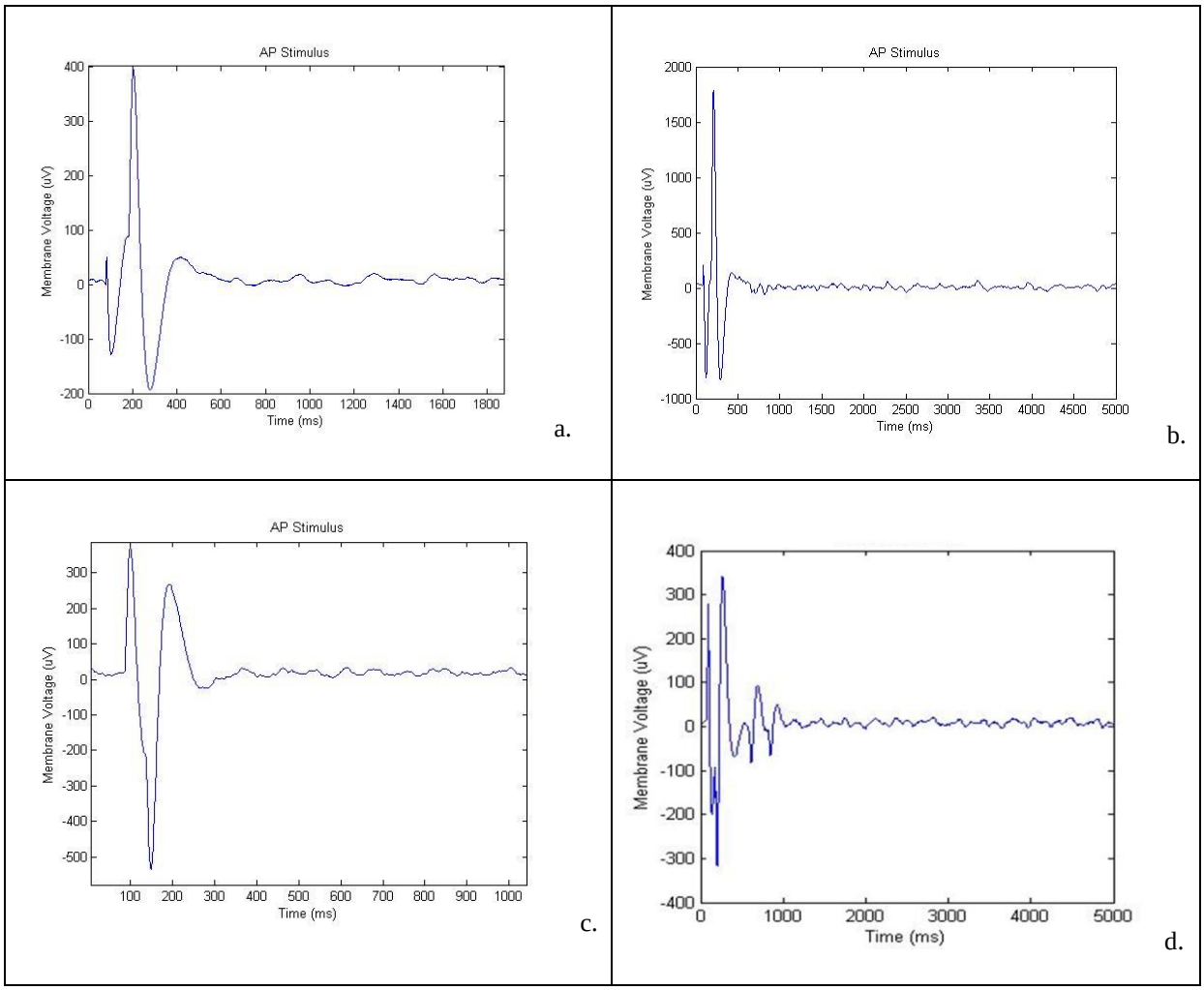


Figure 3: Nerve response to electrical stimulation. Stimulation pulse of 5Vpp, bandwidth: 300Hz/1kHz, sampling rate: 10µs. a,c,d. gain: 100. b. gain: 10000

HFAC and TTX

HFAC waveforms in the ranges of 5-50kHz, 0.1-10mA were applied to nerves, in which a stimulus artifact is seen (Figure 4). TTX was also administered in 4 doses of 10µl of 5nM to observe the effects on propagation of action potentials (Figure 5c-j). A baseline recording from recording electrodes 1 and 2 are taken prior to injecting the nerve with TTX (Figure 5a,b).

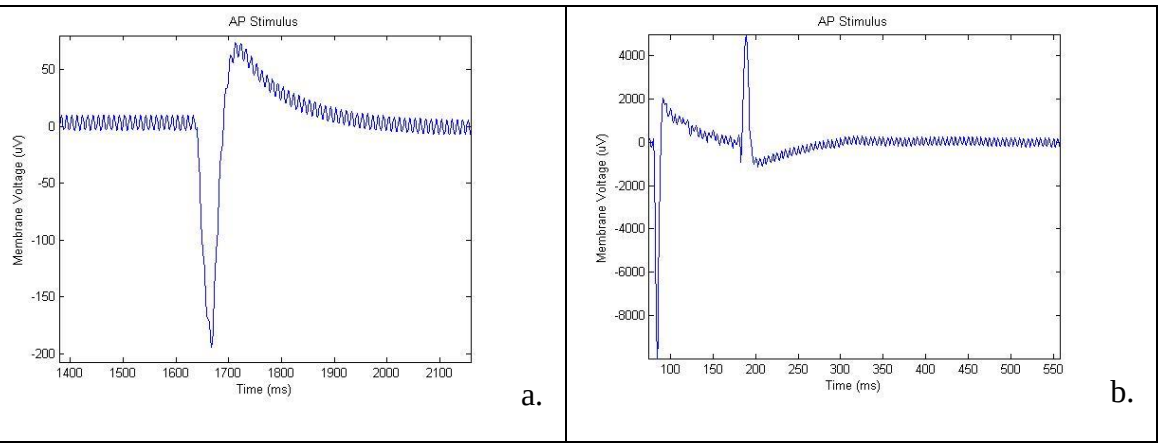
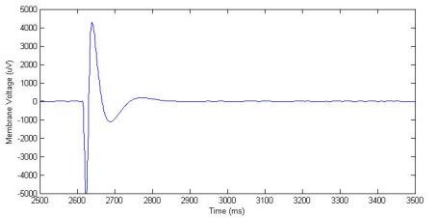
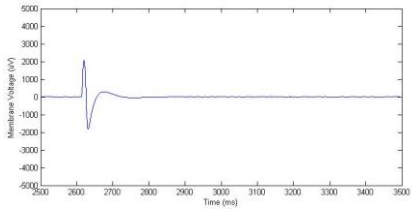
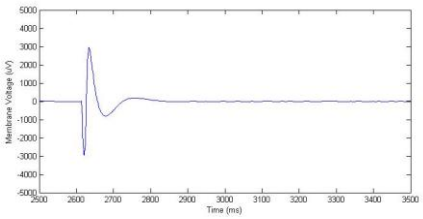
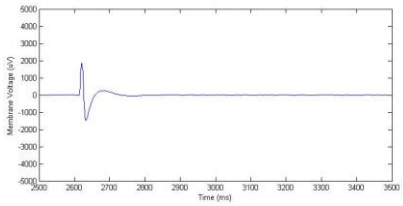
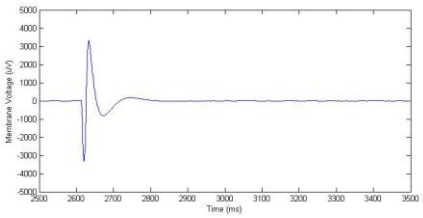
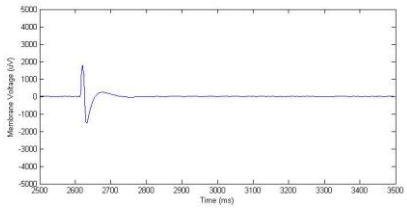
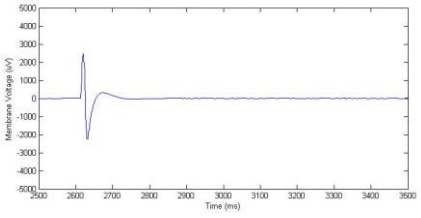
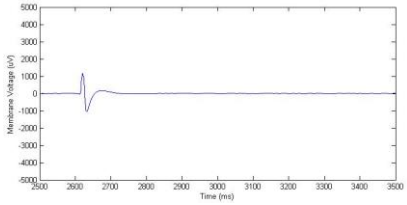


Figure 4: Stimulus artifact in HFAC experiments. Stimulation pulse of 5Vpp, HFAC waveform: 1mA, 1kHz, bandwidth: 300Hz/20kHz, sampling rate: 10µs. a. gain: 100. b. gain: 1000

	Voltage recording 1	Voltage recording 2
Baseline	 a.	 b.
Dose 1	 c.	 d.
Dose 2	 e.	 f.
Dose 3	 g.	 h.

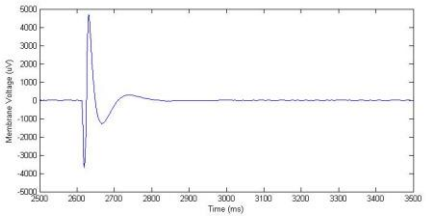
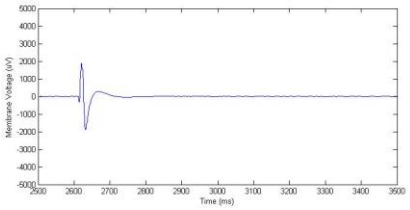
Dose 4	 i.	 j.

Figure 5: Response to nerve with application of TTX. Each dose is 10µl of 5nM TTX. Stimulation pulse of 5Vpp, bandwidth: 300Hz/1kHz, sampling rate: 10µs, gain: 100.

CHAPTER 4

CONCLUSIONS

General Conclusions

Preliminary experiments were conducted to observe the effects of TTX on HFAC block. Compound activity was elicited from nerves using electrical stimulation. However, blocking studies were inconclusive.

When a stimulus pulse is applied to nerves, the stimulus artifact can be seen followed by a nerve response with relatively consistent amplitudes of 300-400μV with a gain of 100. The rat sciatic nerve consists of a mixture of A and C-fibers which can be stimulated by the electrical pulse. However, in the recordings we only see A-fiber responses which are approximately 10 fold the amplitude of C-fibers. The absence of C-fiber responses may be due to it being hidden in the noise present in recordings.

There were some issues faced in conducting HFAC experiments where a nerve response was not elicited before the application of HFAC (Figure 4). Therefore, it is not possible to determine whether action potential propagation was blocked with the HFAC waveform applied. In TTX experiments, the two recording electrodes show CAPs. There is no significant decrease in amplitude for doses 1-4 of nerve responses from the baseline recording (Figure 5). We expected to observe an increasing reduction in CAP amplitude with increasing doses, which differs from the trend of the results from four doses. This could be due to using lower doses of TTX than required to see the effects of sodium channels being blocked. Additionally, TTX may not have been applied within the fascicles of nerves but rather in the surrounding media, thereby decreasing its concentration and effect on nerves. Ultimately, the goal of this study was to examine the effects of increasing doses of TTX on the HFAC block threshold required to cause nerve

propagation block. However, further studies are currently required to determine the effects of TTX on HFAC block thresholds.

Complications

Some issues were faced during the experimentation process that were addressed including nerve viability and ensuring a clear recording of A and C-fiber responses. At first, nerves were stored in a vial filled with Ringer's solution at 37°C for up to one week after being extracted before experiments took place. However, upon taking recordings from the nerve at different time points after being extracted, we determined that the nerve response was most prominent directly following the removal from the rat. Consequently, we conducted experiments directly following the extraction. In order to achieve more consistent and clear CAP recordings, we increased the signal to noise ratio by ensuring all ground electrodes were placed correctly, suction on the nerve was properly set-up, and no wires were touching (Figure 2b,c). Additionally, in order to see both the stimulus artifact and nerve response from the electrical stimulation without overlap, we maintained a larger separation between the stimulating and recording electrode.

Future Studies

Blocking experiments were not conclusive in this experiment. Therefore, additional experiments are needed to determine the role of sodium channels in the HFAC block mechanism. First, accurate and consistent recordings should be taken of HFAC blocking the propagation of action potentials, followed by finding a range of TTX concentrations that alter the threshold for HFAC block. Modulation of HFAC block using a sodium channel blocker, TTX, can elucidate the role of sodium channels in HFAC block. Future studies using other known chemical blockers such as charybotoxin, and

tetraethylammonium can be performed to determine the role of other voltage gated channels in the HFAC blocking mechanism.

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